Chlamydia pneumoniae Does Not Increase Atherosclerosis in the Aortic Root of Apolipoprotein E–Deficient Mice

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Abstract—In epidemiological studies, an association between cardiovascular disease and Chlamydia pneumoniae (C pneumoniae) infection has been observed. Although C pneumoniae has been shown to be present in atherosclerotic lesions, a causal relationship between C pneumoniae infection and atherosclerosis has not been demonstrated. To study this question, we used 2 strains of apolipoprotein (apo) E–deficient mice. Eight-week-old mice on an FVB background that were maintained on either a low- or a high-fat diet were infected 3 times at 1-week intervals with C pneumoniae, and atherosclerotic lesions were measured in the aortic root at 10 weeks after the primary infection. In each of the diet groups, no difference in the extent of atherosclerosis could be observed between the C pneumoniae–infected and control animals. In further studies, 2 strains of apoE-deficient mice (FVB or C57BL/6J background) were infected 4 times at 3- to 4-week intervals, and the extent of atherosclerosis was analyzed 18 weeks later. The mice were kept on either a low- or a high-fat diet. The high-fat diet increased atherosclerosis, and a difference in atherosclerosis susceptibility between the mouse strains was observed. However, C pneumoniae infection did not influence lesion size in either mouse strain. On the other hand, C pneumoniae could not be demonstrated by polymerase chain reaction in any of the atherosclerotic lesions of the infected animals studied. A small decrease in serum cholesterol and triglyceride levels 3 days after the primary infection occurred, but after that no differences in serum lipid levels compared with those in noninfected animals were evident. In the myocardium of C pneumoniae–infected mice, no inflammatory signs could be observed. We conclude that under the experimental conditions used, C pneumoniae infection does not accelerate atherogenic changes in the aortic root of apoE-deficient mice. (Arterioscler Thromb Vasc Biol. 2001;21:578-584.)

Key Words: C pneumoniae infection • atherosclerosis • apoE-deficient mice • animal model

Coronary heart diseases (CHDs) are the leading cause of mortality in Western societies. Atherosclerosis is associated with several risk factors, including smoking, hypertension, dyslipidemia, diabetes, positive family history, male sex, and age. However, only ≈50% of the CHD risk can be explained by these factors, and therefore, other risk factors must contribute.1 One risk factor that has been proposed is chronic infection.2 At present, several lines of evidence suggest that atherosclerosis may be regarded as a chronic inflammatory disease and that infections may play an important role in perpetuating the inflammatory status.

One pathogen that has been implicated to strongly influence atherogenesis is Chlamydia pneumoniae (C pneumoniae) (reviewed by Muhlestein3). This obligate, intracellular bacterium is responsible for a significant proportion of upper and lower respiratory infections, including ≈10% of all pneumonia in adults and ≈5% of bronchitis and sinusitis.4 The prevalence of C pneumoniae antibodies in the population ranges from 50% at the age of 20 years to almost 80% in old age.5 Besides causing acute inflammatory diseases, C pneumoniae has been found to be associated with a number of chronic diseases, including asthma, chronic obstructive pulmonary disease, and reactive arthritis.4 Several epidemiological studies have demonstrated the association of CHD and elevated plasma C pneumoniae antibody levels (see reviews5,6,7). However, this association has not been confirmed in all studies.8–12 In prospective studies, only sparse or no evidence for the association has been obtained.13–16

The observation that C pneumoniae can be detected in atheromatous plaques further strengthens the assumption that C pneumoniae is a causative agent of atherogenesis.17 C pneumoniae has been shown to be present in almost 60% of atherosclerotic lesions compared with 3% in the normal vessel wall.18

The drawback with both epidemiological studies and with those detecting C pneumoniae in the lesions is that neither can determine whether C pneumoniae really has a role in...
atherogenesis or whether it is just an innocent bystander in the lesions. Studies with antibiotics have been performed but so far, only in small populations.\textsuperscript{18,20} However, from those studies it is difficult to conclude whether the results obtained are due to an antimicrobial effect or to nonspecific, anti-inflammatory effects. Large antibiotic trials are underway,\textsuperscript{19,21} but still the crucial question as to whether bacterial infection really has a causal role in atherogenesis or in lesion stability cannot be answered by those kinds of studies.\textsuperscript{22}

To test whether there exists a causal relationship between \textit{C pneumoniae} infection and atherosclerosis, we performed studies in which apolipoprotein E (apoE) –deficient mice were used as a model for atherosclerosis. The causal relationship is not necessarily the same in humans as it is in animals, but at least so far, the effects of different factors on atherosclerosis in mice have been similar to what would be predicted in humans.\textsuperscript{23} This hyperlipidemic animal model can spontaneously develop atherosclerotic lesions that, based on their morphology, are very similar to those in humans. These mice were repeatedly infected with \textit{C pneumoniae}, and the initiation and progression of atherosclerosis were analyzed in the aortic root. Both sexes of mice of 2 different genetic backgrounds were used in combination with low- and high-fat diets.

**Methods**

**Mice**

ApoE-deficient mice on FVB and C57BL/6J backgrounds were used. FVB mice were kindly provided by Prof J.L. Breslow of Rockefeller University, New York, NY, and C57BL/6J mice were obtained from the Jackson Laboratories (Bar Harbor, Me). Mice were housed individually in filter-top cages and fed with either regular low-fat chow (Purina) or a high-fat diet (21% wt/wt fat, 0.15% wt/wt cholesterol, 19.5% wt/wt casein, and no sodium cholate; Harlan Teklad TD 88137) ad libitum. Both male and female mice were used. The animal experiments used in this article were approved by the ethics committee of the University of Tampere.

**Inoculation**

At 8 weeks of age, the mice were mildly sedated with methoxyflu- ran and inoculated intranasally with the \textit{C pneumoniae} Kajaani 7 strain in a volume ranging from 20 to 40 \textmu L in sucrose-phosphate–glutamic acid (SPG) chlamydial transport medium. To determine the influence of \textit{C pneumoniae} on serum lipid levels, 1 set of mice was infected once with 3\times10^6 inclusion-forming units (IFU) of \textit{C pneumoniae} and killed at certain time points for lipid analysis. In the first experiment to study the effect of \textit{C pneumoniae} on atherosclerosis, the mice were inoculated with 3\times10^6 IFU of \textit{C pneumoniae} 3 times at 1-week intervals and killed 10 weeks later. In the second experiment, the mice were infected 4 times at 3- to 4-week intervals and killed 18 weeks later. In this experiment, mice were first infected with 1\times10^6 IFU of \textit{C pneumoniae}, but because \textapprox30\% of the infected mice died a few days after infection and the rest of them looked very sick for a few days, later infections were performed with 1\times10^5 IFU of \textit{C pneumoniae}. As controls, mice were inoculated with the same amount of SPG by following the protocol used with those receiving \textit{C pneumoniae}–specific nested primers in a touchdown polymerase chain reaction (PCR) as described by Tong and Silles.\textsuperscript{26} Amplified products were detected by agarose gel electrophoresis and ethidium bromide staining, as well as by Southern blotting and hybridization with a digoxigenin-labeled probe.

**Blood Collection**

When the mice were killed, 500 to 1000 \textmu L of blood was collected. The blood collections were performed in the morning with the animals having free access to food. Serum samples were fractionated by size-exclusion chromatography on Superose 6 HR gel-filtration columns, with 2 columns (Pharmacia) connected in tandem. Gel-filtration buffer was PBS, pH 7.4. Pooled serum samples (250 \mu L) were applied to the equilibrated column at a flow rate of 0.5 mL/min, and 0.5-mL fractions were collected for further lipid analysis. Serum total cholesterol and triglycerides as well as their concentrations in the gel-filtration fractions of plasma were measured enzymatically with the use of commercial kits (cholesterol, Boehringer Mannheim catalog No. 236691; triglycerides, GPO-Trinder, Sigma catalog No. 337-B).

**Quantitative Atherosclerosis Measurements**

Mice were killed (with CO\textsubscript{2}) and then perfused with saline. Hearts were fixed in 10\% phosphate-buffered formaldehyde. For the quantitative lesion assay, hearts were embedded in 25\% gelatin and cryostat-sectioned at 10- to 12-\mu m thickness, and processing and staining of the aortic root were carried out according to methods previously described by Paigen et al.\textsuperscript{23} Quantification of lesion areas in the aortic root was performed as described earlier.\textsuperscript{25-27} The sections were evaluated for oil red O–staining areas by capturing the images from a camera attached to a light microscope and displayed on a computer monitor. Image analysis was performed with Image-Pro Plus software, version 3.0, in the first experiment and version 4.0 in the second experiment (Medio Cybernetics).

**PCR Analysis**

DNA was extracted from murine aortic tissues by proteinase K lysis and phenol-chloroform extraction, followed by ethanol precipitation. The extracted DNA was amplified by \textit{C pneumoniae}–specific nested primers in a touchdown polymerase chain reaction (PCR) as described by Tong and Silles.\textsuperscript{26} Amplified products were detected by agarose gel electrophoresis and ethidium bromide staining, as well as by Southern blotting and hybridization with a digoxigenin-labeled probe.

**Histology Assay**

Separate heart sections were stained with hematoxylin and eosin for the presence of inflammation in the heart muscle.\textsuperscript{29}
TABLE 1. Lipid Levels in ApoE-Deficient Mice on an FVB Background

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol, mmol/L</th>
<th>Triglycerides, mmol/L</th>
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<td></td>
<td>n</td>
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<tr>
<td>Low-fat diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C pneumoniae</td>
<td>10</td>
<td>16.14±1.98</td>
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<tr>
<td>Control</td>
<td>10</td>
<td>13.12±1.43</td>
</tr>
<tr>
<td>High-fat diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C pneumoniae</td>
<td>4</td>
<td>56.61±8.89*</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>57.94±9.58*</td>
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Serum total cholesterol and triglycerides in apoE-deficient male mice on an FVB background maintained on low-fat chow and high-fat diets for 10 weeks were measured after 3 repeated inoculations with C pneumoniae or control SPG buffer. Data are mean±SD, where n=number of animals.

*P<0.005 when compared with low-fat diet.

Statistical Analysis

Plasma lipids are reported as mean±SD. Comparisons between groups were made with Student's 2-tailed t test.

Results

To study the effect of C pneumoniae infection on serum lipid levels, we used apoE-deficient mice on an FVB background, and the mice were maintained on the (low-fat) chow diet. One group was infected once with 3×10⁶ IFU of C pneumoniae while the other served as a control. At each time point, 4 to 11 infected and control mice were used (Figure 1). Before infection, serum cholesterol values were similar in both groups (12.76±2.05 mmol/L in the infected group vs 11.98±1.89 mmol/L in controls; P=NS). C pneumoniae infection caused a small drop in total cholesterol 3 days after the infection (P=0.09; Figure 1A), but thereafter the serum cholesterol levels were similar in infected and noninfected animals. Also at day 3 after infection, a decrease in serum triglycerides was evident (P=0.01). Total triglyceride values differed significantly only 6 weeks (42 days) after C pneumoniae inoculation (5.88±2.32 mmol/L in infected and 3.75±1.28 mmol/L in control animals; P=0.02). The reduction in total cholesterol and triglyceride values 3 days after infection was due to a drop in the VLDL fraction (data not shown). Similar results were obtained in experiments in which repeated inoculations with C pneumoniae were used (Table 1). In the groups fed a high-fat diet, serum cholesterol values increased almost 5-fold while serum triglyceride levels were unaffected (Table 2). Also in mice on the high-fat diet, C pneumoniae infection did not cause any difference in plasma lipid levels compared with those in noninfected animals. It can thus be concluded that under the experimental conditions used, C pneumoniae does not significantly affect plasma lipid levels.

To study the effect of C pneumoniae on the progression of atherosclerotic lesions, 2 separate sets of experiments were performed. ApoE-deficient FVB male mice were infected with C pneumoniae 3 times at 1-week intervals between inoculations. Ten control and ten C pneumoniae–infected mice were kept on the chow diet, and 6 control and 4 infected mice were fed the high-fat diet. All C pneumoniae–infected mice had antibodies against C pneumoniae, with titers ranging from 32 to 128 (mean, 96), whereas in the sera from noninfected mice, no antibodies were present. Serum total triglyceride and cholesterol values were not significantly different between C pneumoniae–infected and control mice on either diet (Table 1). Ten weeks after the first inoculation, the mice were killed and the atherosclerotic lesions characterized. In noninfected control mice fed a chow diet, the mean aortic lesion area was 644±2340 μm² while in chow-fed, C pneumoniae–infected mice, the area was 6439±4007 μm² (Figures 2 and 3). In mice fed a high-fat diet, the atherosclerotic lesions were much more pronounced: 33 857±20 376 in the control group and 27 016±5211 μm² in the infected group (Figure 3). However, there was no significant difference in the magnitude of lesion size in C pneumoniae–infected and noninfected control mice on either diet.

In the next set of experiments, 2 strains of apoE-deficient mice, on FVB and C57BL/6J backgrounds, were used (Figures 2 and 4). The animals were inoculated 4 times with 3 to 4 weeks between inoculations and killed 18 weeks after the first inoculation. All C pneumoniae–infected mice had antibodies against C pneumoniae (titers ranging from 32 to 128; mean, 101), but none of the controls did. Five FVB male mice were C pneumoniae infected and 5 served as controls, and all 10 were kept on the high-fat diet. Two infected mice died after the primary infection. The mean lesion areas were 122 022±48 467 and 101 442±49 903 μm² in infected and control mice, respectively (P=NS). Five C57BL/6J male mice (1 died after the primary infection) were infected with C pneumoniae and were kept as controls, and all 10 mice were kept on the chow diet. Five C pneumoniae–infected C57BL/6J mice (4 mice died after the primary infection) and 5 controls were kept on the high-fat diet. The lesion areas in C pneumoniae–infected and control mice maintained on the chow diet were 128 669±33 898 and 129 299±39 783 μm², respectively, and on the high-fat diet, 322 760 and 101 442 μm², respectively.
Atherosclerosis was analyzed in C57BL/6J female mice also. Five *C. pneumoniae*-infected and 5 control female mice were kept on the chow diet and 3 infected and 4 controls, on the high-fat diet. The mean lesion areas for the chow diet–fed group were 251,427 ± 31,114 μm² for infected and 231,924 ± 42,076 μm² for control animals. The corresponding figures for female mice maintained on the high-fat diet were 337,789 ± 39,759 and 378,257 ± 132,111 μm², respectively (Figure 4). The atherosclerotic lesion areas in *C. pneumoniae*-infected and control mice were not significantly different. Serum lipid values did not differ significantly from each other in *C. pneumoniae*-infected and control groups (Table 2). The cholesterol-raising effect of the high-fat diet was clearly displayed in both male and female mice. However, the diet did not affect serum triglyceride levels. Even though atherosclerosis was more extensive in C57BL/6J mice than in FVB mice, on the high-fat diet FVB mice had significantly higher serum total triglyceride and cholesterol levels than did C57BL/6J mice (*P* < 0.0001).

The presence of *C. pneumoniae* DNA was analyzed by PCR in the aortic arch in 20 *C. pneumoniae*-infected mice and 10 control mice. None of the aortic samples were positive for chlamydial DNA (data not shown). The myocardium of *C. pneumoniae*-infected and control mice was also analyzed. The overall morphology was not different in the 2 groups, and no inflammatory reactions, as determined by the absence of clusters of mononuclear inflammatory leukocytes, were observed in the myocardium or in the perivascular area of either control or *C. pneumoniae*-infected mice (data not shown).

**Discussion**

On the basis of epidemiological studies and the localization of *C. pneumoniae* in atherosclerotic lesions, *C. pneumoniae* has been suggested to play a role in the development of atherosclerosis. Whether it influences the initiation or progression of atherogenesis or has a role in the regulation of plaque stability was not answered by those studies. We studied the influence of *C. pneumoniae* in a relevant animal model to follow the development of atherosclerotic lesions. ApoE-deficient mice were infected intranasally with *C. pneumoniae*, and atherosclerotic changes were quantified in the aortic root. The animals were infected repeatedly to cause a
chronic \textit{C. pneumoniae} infection. At first, reinfections were performed at 1-week intervals, but with the evidence that in mice \textit{C. pneumoniae} can be cultivated from lungs up to 4 weeks after infection, later reinfections were performed 3 to 4 weeks apart. With both infection protocols, we could not demonstrate differences in lesion areas between \textit{C. pneumoniae}-infected and control mice. Hu and coworkers demonstrated in LDL receptor–deficient mice that \textit{C. pneumoniae} infection could have an effect on atherosclerosis but only when combined with elevated serum cholesterol. We kept the mice on both the low-fat chow diet and on a high-fat diet and could clearly demonstrate an increase in the degree of atherosclerosis when serum lipids were elevated. However, \textit{C. pneumoniae} infection did not affect the progression of atherosclerosis on either diet. Different mouse strains have been demonstrated to vary in their susceptibility to acquire atherosclerotic lesions, and the same has been observed in apoE-deficient mice on different background strains. We wanted to analyze whether 1 background would be more sensitive than another to the possible atherogenic effect of \textit{C. pneumoniae}. An atherosclerosis-prone background, C57BL/6J, and an atherosclerosis-resistant background, FVB, were chosen. \textit{C. pneumoniae} infection did not, however, accelerate atherosclerosis in either of the 2 strains. Both male and female mice were used, and no effects of \textit{C. pneumoniae} on atherogenesis could be observed in either sex. These findings suggest that \textit{C. pneumoniae} infection is not critical in the initiation or progression of atherosclerosis in this animal model.

Animal models have also been used to analyze the effect of \textit{C. pneumoniae} on atherosclerosis. In rabbits, endothelial intimal thickening, grade III atherosclerotic lesions, and even calcification have been detected in \textit{C. pneumoniae}-infected animals. Furthermore, it has been possible to prevent \textit{C. pneumoniae}-induced intimal thickening by administering antibiotics that are effective against \textit{Chlamydia}. In LDL receptor–deficient mice, \textit{C. pneumoniae} infection was observed to increase atherosclerosis, but only 9 months after the primary infection and on a high-fat diet. The infection did not increase atherosclerosis at 6 months after infection and on the high-fat diet, and no effect was observed 6 or 9 months after infection in conjunction with a low-fat diet. In the present article, the longest time of \textit{C. pneumoniae} exposure was \textless;4 months, and thus, this relatively short period could be 1 plausible explanation for the different findings in these 2 studies. However, mice 9 months of age are already quite old, and the finding with LDL receptor–deficient mice would mean that even though \textit{C. pneumoniae} was present in those mice from the beginning, it affected the process only later in life. Moazed and coworkers used apoE-deficient mice as the animal model for \textit{C. pneumoniae} infection and atherosclerosis. They found that \textit{C. pneumoniae} accelerated the atherosclerotic process significantly. In their experiments, the increase was observed even on a low-fat diet and was already detectable 8 weeks after the initial infection, when the mice were 16 weeks of age. The main difference in their experimental setup compared with ours is the aortic segment where atherosclerosis was quantified: they used the aortic arch and we used the aortic root. It is possible that the atherosclerotic process is different in these 2 locations. However, it has been shown earlier that both of these locations are sites of predilection for lesion development. It is, however, possible that lesion progression is different in the 2 places and that \textit{C. pneumoniae} preferentially influences the process in the aortic arch.

The evidence for \textit{C. pneumoniae} as a potential causative agent for atherosclerosis is based on serological studies and the presence of these bacteria in atherosclerotic lesions. In the current work, we found antibodies to \textit{C. pneumoniae} in all of the infected mice, whereas none of the noninfected control mice had antibodies to \textit{C. pneumoniae}. However, we could not detect \textit{C. pneumoniae} DNA in any of the aortic samples analyzed. \textit{C. pneumoniae} bacterial viability was based on the fact that the animals looked sick for a few days after each infection, and when too many bacteria were accidentally used as an inoculant, as described in Methods, some of the mice died of the infection. Aortic arches as well as aortic roots have been found to be areas with the most pronounced atherosclerotic lesions. Our PCR analysis was done with aortic arch samples, and thus, the negative finding cannot be explained by a lack of lesions in the tissues analyzed. The \textit{C. pneumoniae} strain used in our experiments was different.
from strains used by other groups. In our study, the *C. pneumoniae* strain Kajaani 7 (a Mycoplasma-free Finnish epidemic strain) was used. In the work by Hu and coworkers, the *C. pneumoniae* strain AR-39 was used, and the presence of chlamydial antigens in atherosclerotic lesions was reported. Campbell and coworkers also infected mice with *C. pneumoniae* strain AR-39, and they could detect chlamydial DNA in aortic tissues. Also, the age of the mice infected was observed to be critical in the study by Campbell and coworkers. When the mice were infected at 16 weeks of age, all of the aortas examined contained chlamydial DNA, but when the infection was performed at 8 weeks of age, only part of the aortas examined contained chlamydial DNA. Our mice were all 8 weeks old when they were first infected. However, in the study by Moazed and coworkers, in which *C. pneumoniae* infection increased atherosclerosis, the mice were also infected at the age of 8 weeks. The strain used in their study was AR-39. LDL receptor-deficient mice used by Hu and coworkers were 4 to 5 weeks of age when first infected. Based on these findings, it can be concluded that the presence of antibodies to *C. pneumoniae* does not necessarily mean that the *C. pneumoniae* organism would be present in atherosclerotic lesions, and there could be chlamydial strain-specific differences in their ability to disseminate from lungs to other tissues such as the arterial wall.

In human studies, the initiation and progression of atherosclerosis are difficult to examine, and the presence of atherosclerosis usually becomes evident only after acute vascular events have occurred, ie, when advanced lesions are already present. Most epidemiological studies published thus far have found elevated *C. pneumoniae* antibody titers to be associated with advanced atherosclerotic lesions and acute vascular events with plaque rupture, resulting in acute myocardial infarction or stroke. These studies do not tell much about the role of *C. pneumoniae* infection in atherogenesis but mainly about the final stage, with rupture of the plaque and thrombus formation. Thus, these studies suggest that *C. pneumoniae* infection could have a crucial role in plaque stability. The presence of *C. pneumoniae* in lesions does not prove that it has a role in atherogenesis, *C. pneumoniae* is found in monocyte-macrophages and can be disseminated from the lungs to atheromatous plaques; thus, it could be in the lesions as an innocent bystander. It is possible that when present in the lesion, *C. pneumoniae* causes a chronic inflammation and thus perpetuates atherogenesis, but the presence of the bacterium in the lesion does not prove this hypothesis. Another possible mechanism for the association between *C. pneumoniae* and acute vascular events is that *C. pneumoniae* makes the plaque unstable. *C. pneumoniae* contains lipopolysaccharide and heat shock protein 60, both of which are strong inducers of matrix metalloproteinases, and thus, the presence of *C. pneumoniae* could enhance the action of proteolytic enzymes and predispose towards plaque rupture. Our study was not designed to answer whether *C. pneumoniae* causes atherosclerotic plaques to be unstable but rather to determine whether the infection plays a role in the initiation and progression of atherosclerosis. Mice do not normally have infarctions, even though they develop advanced atherosclerotic lesions, and currently, we do not have a good animal model for infarction. With current animal models, additional manipulations are needed if one wants to test the role of *C. pneumoniae* infection in plaque instability and rupture.

*Chlamydia* infections have also been associated with other types of heart diseases. Bachmaier and coworkers found that an outer membrane protein of *C. pneumoniae* has strong sequence homology with a peptide from murine heart muscle-specific α-myosin heavy chain. Immunization of mice with the peptide from the α-myosin heavy chain induces severe autoimmune heart disease. Immunizing mice with the homologous peptide from *C. pneumoniae* induced a similar reaction, with perivascular inflammation, fibrotic changes, and blood vessel occlusion in the heart. These observations would have occurred through antigenic mimicry. We therefore analyzed the myocardium of control and *C. pneumoniae*-infected mice but could not detect any signs of perivascular inflammation or fibrosis in either group. In Bachmaier’s and our work, different mouse strains were used, and this may help to explain the difference. Also, whereas Bachmaier and coworkers used purified peptides that were infused into mice, in our experiments, whole live *C. pneumoniae* bacteria were delivered intranasally into mice. In the current study, we could not demonstrate that intranasally administered *C. pneumoniae* infection caused inflammatory reactions in the heart muscle of apoE-deficient mice.

In conclusion, our experiments do not demonstrate any effect of *C. pneumoniae* infection on the initiation or progression of atherosclerosis in apoE-deficient mice. Although seroconversion was obvious in all mice after infection with *C. pneumoniae*, no *C. pneumoniae* DNA could be demonstrated in aortic tissues. It is possible that the *C. pneumoniae* strain used in our experiment does not disseminate from the respiratory system. It is also possible that the age of primary infection could be critical for the bacteria to disseminate from the lungs. Our experiments demonstrate that the presence of antibodies to *C. pneumoniae* does not mean that the bacteria can also been found in the arterial wall. Further human and animal studies are needed to determine the molecular mechanisms underlying the reported association between *C. pneumoniae* infection and atherosclerotic disease.

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**References**


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